ANTICANCER RESEARCH 17: 113-116 (1997)

Interleukin-6 Production in Primary Histoculture by Normal Human Kidney and Renal Tumor Tissues

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Abstract. *Interleukin-6 (IL-6) is a multifunctional cytokine with many biologic activities in vitro, including synergistic or antagonistic actions with one or more other cytokines. The role and induction parameters of IL-6 in renal cell carcinoma (RCC) are not fully understood. To understand the ability of RCC to produce IL-6, we determined the IL-6 concentration in the supernatant of histocultured human normal kidney, RCC, renal Wilms' tumor and renal oncocytoma. From these studies, we conclude that the kidney is one of the main sources of IL-6. Normal renal cortical tissues and renal tumors can produce IL-6 in histoculture without stimulation. Thus histoculture supports the long-term production of IL-6, potentially allowing many important studies of this cytokine in the normal and malignant kidney.*

Interleukin-6 (IL-6) can be produced by various cell types including fibroblasts, monocytes/macrophages, T cells, B cells, endothelial cells, epidermal cells, synovial cells, keratinocytes and diverse tumor cells (4). Tsukamoto *et al* (7) suggested that some renal cell carcinomas can produce IL-6 and this cytokine is responsible for several paraneoplastic syndromes in this carcinoma. Hamao *et al* (3) reported that IL-6 plays an important role in the progression of renal cell carcinoma and that measurement of IL-6 in patients with renal cell carcinoma may be useful clinically. There have been reports that IL-6 is an autocrine growth factor for renal cell carcinoma, but this is highly controversial. In addition, recombinant human IL-6 has clear antitumor activity in a mouse tumor model with no demonstrable toxicity (6).

A previous report has suggested that a human renal cell carcinoma cell line is capable of producing IL-6 *in vitro* and that IL-6 production may be related to an elevation of acute phase proteins in patients with renal cell carcinoma (5). However, all of these studies were carried out with cell lines. Chang *et al* (1,2) have reported the three-dimensional histoculture of human renal cortical tissues on collagen sponge gels. We herein report that the production of IL-6 in histocultured normal and malignant human kidney tissues.

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Key Words: Interleukin-6, histoculture, human kidney, renal tumor.

Materials and Methods

Human renal tumor tissues, normal human renal cortical tissues, Wilms' tumor and renal oncocytoma, were transported in a sterile container to the laboratory. All surgical specimens were divided into 2 to 3 mm diameter pieces and five pieces were placed on top of previously hydrated Spongostans gel (1 × 1 × 1 cm, Health Design Indust. Rochester NY U.S.A.). Each gel occupied one well of a six-well plate. Three milliliters of minimal essential medium supplemented with 10 % fetal bovine serum, 50 μg/ml gentamicin and cefotaxime at a final concentration of 1 μg/ml were added to each well. The final volume of medium was sufficient to reach the upper gel surface without immersing it. Covered culture plates were maintained in a humidified, 5% CO_2 incubator at 37°C. The culture underwent sterile media changes every 72 hours.

Histoculture was continued up to 4 periods. Each period consisted of 3 days. Autoradiography was carried out after the cultures were exposed to medium containing 4 μCi/ml [^{3}H]thymidine for 72 hours. Fixation, embeding, sectioning and deparaffinization were subsequently carried out. Specimen slides were exposed to Kodak NTB2 liquid emulsion for 10 days at 4°C followed by standard development in D19 Kodak developer. The slides were then stained with hematoxylin and eosin. The DNA labeling index is defined as the number of cells labeled per total cells evaluated (400 cells minimum). An effort was made to evaluate only the most active areas of all slides to avoid false-positive scoring.

To determine the amount of IL-6 secreted into the culture supernatant, collagen gel supported histoculture media were collected every 72 hours for 4 periods. The IL-6 concentration was measured using an sandwich enzyme immunoassay (R and D Systems Minneapolis, MN, U.S.A.) according to recommended procedure. The limit of detection of the test was 3.1 pg/ml.

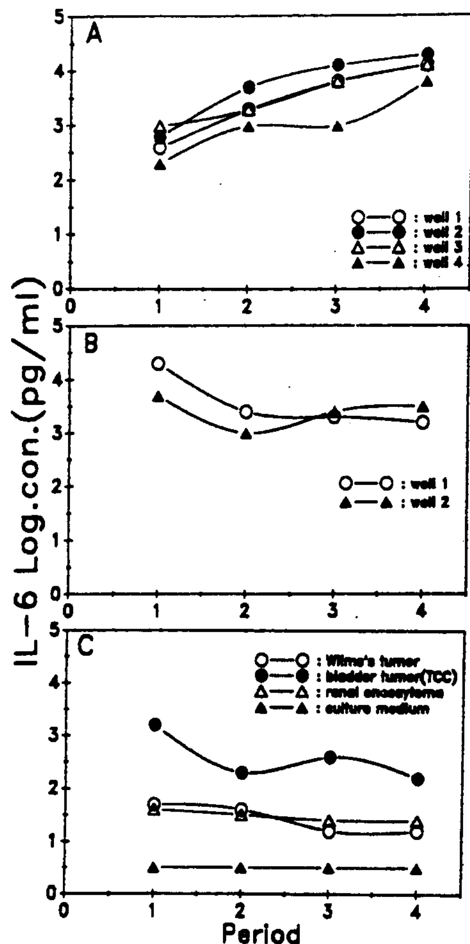

Figure 1. *IL-6 production in normal and malignant kidney. A: The IL-6 content was measured from the conditioned medium of histocultured human renal cortical tissues. IL-6 production increased in continuous serial histoculture. B: The IL-6 content was measured from the conditioned medium of histocultured human renal cell carcinoma. Measurments were made every 72 hours at each medium change (one period). C: The IL-6 content was measured from the conditioned medium of histocultured human renal Wilms' tumor, bladder transitional cell carcinoma and renal oncocytoma. The minimum detectable amount of IL-6 was 3.1 pg/mL*

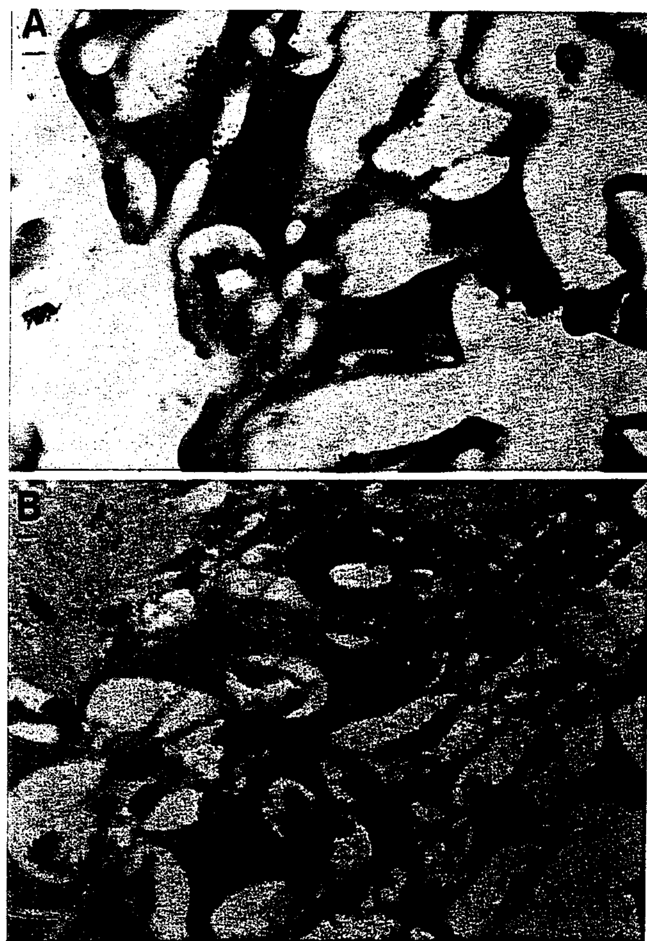

Figure 2. *[^{3}H]Thymidine incorporation in histocultured normal and malignant kidney. A: [^{3}H]thymidine incorporation in human renal cortical tissue noted by grains in the autoradiogram. IL-6 production as shown in Figure 1 and correlated with [^{3}H]thymidine incorporation note that the human renal cortical tissue formed new tubules demonstrated by [^{3}H]thymidine incorporation. B: Histocultured renal cell carcinoma with significant [^{3}H]thymidine uptalce as demonstrated by the grains in the autoradiogram. IL-6 production as shown in Figure 1 correlated with the [^{3}H]thymidine incorporation.*

Results and Discussion

The human normal kidney tissue, renal cell carcinoma, Wilms' tumor, renal oncocytoma and bladder tumor were histocultured for up to 4 periods. The IL-6 content of the medium was measured in 4 different wells of histocultured human renal cortical tissues. IL-6 production in the four wells measured in the first period was 390, 580, 1000 and 195 pg/ml, respectively. Production of IL-6 increased sharply during histoculture. The IL-6 production in the four wells measured in the fourth period was 12,800, 20,800, 12,800, and 6,080 pg/ml, respectively (Figure 1A).

The IL-6 content of the medium was measured in 2 different wells of histocultured human renal cell carcinoma. Supernatant IL-6 concentrations of during the first period were 20,800 and 5,600 pg/ml, respectively. The IL-6 production, however, slightly decreased in histoculture by the fourth period. In the first well, the IL-6 concentrations were 20,800, 2,610, 1,850 and 1,750 pg/ml in periods 1-4, respectively. In the second the well IL-6 concentrations were 5,100, 1,000, 2,610 and 2,850 pg/ml in periods 1-4, respectively (Figure 1B).

IL-6 production was measured in histocultured human renal Wilms's tumor, renal oncocytoma, bladder transitional

cell carcinoma as described above. The IL-6 production was minimal in the Wilms' tumor and renal oncocytoma. In the Wilms' tumor, the IL-6 concentrations were 45, 36, 16 and 16 pg/ml in periods 1-4, respectively. In the renal oncocytoma, the IL-6 concentrations were 36, 29, 24 and 27 pg/ml in periods 1-4, respectively. The histocultured bladder tumor produced more IL-6 than the Wilms' tumor and the renal oncocytoma, ranging from 160 to 1,720 pg/ml during periods 1 and 4, respectively. It was, however, lower than histocultured renal cell carcinoma or renal cortical tissues. There was no detectable IL-6 in fresh histoculture medium (Figure 1C).

[^{3}H]Thymidine autoradiography was carried out on the histocultured specimens. IL-6 production was closely correlated with the degree of [^{3}H]thymidine incorporation of both of the renal cell carcinoma and renal cortical tissues. Histocultured renal cortical tissues also demonstrated newly formed renal tubules which were incorporating with [^{3}H]thymidine (Figure 2).

From these histoculture studies, we conclude that the normal and malignant kidney is one of the main sources of IL-6 production. Histoculture supports the relatively long-term production of IL-6 by these tissues, allowing many future studies of this important cytokine.

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Received July 25, 1996
Accepted September 9, 1996